

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
11 March 2004 (11.03.2004)

PCT

(10) International Publication Number
WO 2004/020662 A3

(51) International Patent Classification⁷: **C12Q 1/68**

(21) International Application Number:
PCT/EP2003/007827

(22) International Filing Date: 18 July 2003 (18.07.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10239313.3 27 August 2002 (27.08.2002) DE
10255104.9 26 November 2002 (26.11.2002) DE

(71) Applicant (for all designated States except US): **EPIGENOMICS AG** [DE/DE]; Kastanienallee 24, 10435 Berlin (DE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **MAIER, Sabine** [DE/DE]; Markelstrasse 60, 12163 Berlin (DE).

(74) Agents: **KRAUSS, Jan et al.**; Boehmert & Boehmert, Pettenkoferstrasse 20-22, 80336 München (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,

CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(88) Date of publication of the international search report:
26 August 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD AND NUCLEIC ACIDS FOR THE ANALYSIS OF BREAST CELL PROLIFERATIVE DISORDERS

(57) Abstract: The present invention relates to modified and genomic sequences, to oligonucleotides and/or PNA-oligomers for detecting the cytosine methylation state of genomic DNA, as well as to a method for ascertaining genetic and/or epigenetic parameters of genes for use in the differentiation, diagnosis, treatment and/or monitoring of breast cell proliferative disorders, or the predisposition to breast cell proliferative disorders.

WO 2004/020662 A3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/07827

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, Sequence Search, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/059347 A (UNIV JOHNS HOPKINS MED) 1 August 2002 (2002-08-01) claims 1-44	1,2, 12-38
Y	WO 02/38801 A (GRIGG GEOFFREY WALTER ;MOLLOY PETER (AU); HUMAN GENETIC SIGNATURES) 16 May 2002 (2002-05-16) claims 1-16 ----- -/--	1,2, 12-38

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

11 February 2004

Date of mailing of the international search report

27. 05. 2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Gabriels, J

INTERNATIONAL SEARCH REPORT

 Internat Application No
 PCT/EP 03/07827

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GITAN RAAD S ET AL: "Methylation-specific oligonucleotide microarray: a new potential for high-throughput methylation analysis" GENOME RESEARCH, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 12, no. 1, January 2002 (2002-01), pages 158-164, XP002208051 ISSN: 1088-9051 the whole document	1,2, 12-38
Y	----- YAN P S ET AL: "Dissecting complex epigenetic alterations in breast cancer using CpG Island microarrays" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 61, no. 23, 1 December 2001 (2001-12-01), pages 8375-8380, XP002243660 ISSN: 0008-5472 the whole document	1,2, 12-38
Y	----- HUANG TIM HUI-MING ET AL: "Methylation profiling of CpG islands in human breast cancer cells" HUMAN MOLECULAR GENETICS, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 8, no. 3, March 1999 (1999-03), pages 459-470, XP002175857 ISSN: 0964-6906 the whole document	1,2, 12-38
Y	----- DE IORIO P ET AL: "Implication of laminin and collagen type IV expression in the progression of breast carcinoma." ANTICANCER RESEARCH. GREECE 2001 MAR-APR, vol. 21, no. 2B, March 2001 (2001-03), pages 1395-1399, XP008027518 ISSN: 0250-7005 the whole document	1,2, 12-38
X	----- WO 01/40521 A (CURAGEN CORP ; LEACH MARTIN (US); SHIMKETS RICHARD A (US)) 7 June 2001 (2001-06-07) Human coding sequence polymorphic site SEQ ID NO: 544. Claim 1; Page 260; SEQ ID NO: 542. Claim 1; Page 260; SEQ ID NO: 540. Claim 1; Page 259 page 260; claim 1	4-11
X	----- WO 01/29262 A (POHL MARK ; ORCHID BIOSCIENCES INC (US); PICOULT NEWBURG LESLIE (US) 26 April 2001 (2001-04-26) SNP specific lower PCR primer SEQ ID 3530. Claim 1; Page 68claim 1	3-11
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/07827

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/77373 A (PIEPENBROCK CHRISTIAN ;BERLIN KURT (DE); EPIGENOMICS AG (DE); OLEK) 18 October 2001 (2001-10-18) the whole document	3-11
P,X	----- WO 02/070742 A (EPIGENOMICS AG) 12 September 2002 (2002-09-12) the whole document	1-38
Y	----- US 5 744 305 A (FODOR STEPHEN P A ET AL) 28 April 1998 (1998-04-28) cited in the application the whole document -----	12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/07827

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: ³⁹
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Invention 1: claims 1-38 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims 24 - 35 include the step of obtaining a biological sample containing genomic DNA and are therefore directed to a diagnostic method practised on the human/animal body. The search has been carried out and based on the differential methylation state of genes in breast cell proliferative disorders and the detection thereof.

Continuation of Box I.1

Claims Nos.: 39

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-38 (all partially)

Isolated nucleic acids, nucleic acids or peptide nucleic acids oligomers derived from SEQ ID NO:1 (COL4A2 gene), sets thereof and arrays or kits comprising them for the use in methods for differentiating between breast cell proliferative disorders and normal tissue based on the methylation state.

Invention 2-73: claims 1-38 (all partially)

Idem as invention 1 but limited to the sequences having SEQ ID NO:2-73 (Invention 2 is limited to SEQ ID NO:2; Invention 3 is limited to SEQ ID NO:3; ...; Invention 73 is limited to SEQ ID NO:73)

Invention 74: claims 1-38 (all partially)

Isolated nucleic acids, nucleic acids or peptide nucleic acids oligomers derived from SEQ ID NO:366 (Calcitonin gene), sets thereof and arrays or kits comprising them for the use in methods for differentiating between breast cell proliferative disorders and normal tissue based on the methylation state.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/07827

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02059347	A	01-08-2002	US 2003017454 A1	23-01-2003
			WO 02059347 A2	01-08-2002
			US 2003138783 A1	24-07-2003

WO 0238801	A	16-05-2002	WO 0238801 A1	16-05-2002
			AU 1481102 A	21-05-2002
			EP 1337662 A1	27-08-2003
			US 2004086944 A1	06-05-2004

WO 0140521	A	07-06-2001	AU 1940001 A	12-06-2001
			CA 2392948 A1	07-06-2001
			EP 1235936 A2	04-09-2002
			JP 2004508003 T	18-03-2004
			WO 0140521 A2	07-06-2001

WO 0129262	A	26-04-2001	AU 1085401 A	30-04-2001
			WO 0129262 A2	26-04-2001

WO 0177373	A	18-10-2001	DE 10019058 A1	20-12-2001
			AU 4835201 A	24-09-2001
			AU 5038101 A	24-09-2001
			AU 5478801 A	23-10-2001
			AU 5479401 A	23-10-2001
			AU 7384001 A	23-10-2001
			AU 7566301 A	23-10-2001
			AU 7633001 A	23-10-2001
			AU 7633101 A	23-10-2001
			AU 7748701 A	23-10-2001
			AU 7842001 A	07-11-2001
			AU 8960001 A	11-12-2001
			WO 0177373 A2	18-10-2001
			DE 20121960 U1	15-01-2004
			DE 20121964 U1	15-01-2004
			DE 20121965 U1	24-12-2003
			DE 20121968 U1	18-12-2003
			DE 20121969 U1	24-12-2003
			DE 20121970 U1	24-12-2003
			DE 20121972 U1	24-12-2003
			DE 20121973 U1	24-12-2003
			DE 20121974 U1	24-12-2003
			DE 20121977 U1	24-12-2003
			WO 0168911 A2	20-09-2001
			WO 0168912 A2	20-09-2001
			WO 0177375 A2	18-10-2001
			WO 0177164 A2	18-10-2001
			WO 0177376 A2	18-10-2001
			WO 0177377 A2	18-10-2001
			WO 0181622 A2	01-11-2001
			WO 0192565 A2	06-12-2001
			WO 0177378 A2	18-10-2001
			WO 0176451 A2	18-10-2001
			EP 1283905 A2	19-02-2003
			EP 1268855 A2	02-01-2003
			EP 1268857 A2	02-01-2003
			EP 1272670 A2	08-01-2003
			EP 1278892 A1	29-01-2003
			EP 1278893 A2	29-01-2003
			EP 1274865 A2	15-01-2003

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/07827

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0177373	A		EP 1274866 A2	15-01-2003
			EP 1360319 A2	12-11-2003
			EP 1370685 A2	17-12-2003
			EP 1268861 A2	02-01-2003
			JP 2004507213 T	11-03-2004
			JP 2004507214 T	11-03-2004
			JP 2004508807 T	25-03-2004
			JP 2003534780 T	25-11-2003
			JP 2003531589 T	28-10-2003
			US 2004029123 A1	12-02-2004
WO 02070742	A	12-09-2002	CA 2439854 A1	12-09-2002
			WO 02070742 A1	12-09-2002
			EP 1364069 A1	26-11-2003
			US 2002137086 A1	26-09-2002
US 5744305	A	28-04-1998	US 5489678 A	06-02-1996
			US 6406844 B1	18-06-2002
			US 5445934 A	29-08-1995
			US 5405783 A	11-04-1995
			US 5143854 A	01-09-1992
			US 2003119008 A1	26-06-2003
			US 6600031 B1	29-07-2003
			US 6346413 B1	12-02-2002
			US 6566495 B1	20-05-2003
			US 6310189 B1	30-10-2001
			US 2002155588 A1	24-10-2002
			US 2003082831 A1	01-05-2003
			US 5889165 A	30-03-1999
			US 5753788 A	19-05-1998
			US 6491871 B1	10-12-2002
			US 2002064796 A1	30-05-2002
			US 2003013100 A1	16-01-2003
			US 6329143 B1	11-12-2001
			US 2002192693 A1	19-12-2002
			US 2003064391 A1	03-04-2003
			US 6261776 B1	17-07-2001
			US 2003235853 A1	25-12-2003
			US 6291183 B1	18-09-2001
			US 6225625 B1	01-05-2001
			US 6403957 B1	11-06-2002
			US 5510270 A	23-04-1996
			AT 110738 T	15-09-1994
			AT 175421 T	15-01-1999
			AU 651795 B2	04-08-1994
			AU 5837190 A	07-01-1991
			AU 672723 B2	10-10-1996
			AU 7765594 A	04-05-1995
			BR 9007425 A	21-07-1992
			CA 2054706 A1	08-12-1990
			CA 2278878 A1	08-12-1990
			CA 2278883 A1	08-12-1990
			CA 2391491 A1	13-12-1990
			DE 69012119 D1	06-10-1994
			DE 69012119 T2	22-12-1994
			DE 69032888 D1	18-02-1999
			DE 69032888 T2	29-07-1999
			DE 953835 T1	13-06-2002

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/07827

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5744305	A	DK 476014 T3	14-11-1994
		DK 619321 T3	30-08-1999
		EP 0476014 A1	25-03-1992
		EP 0619321 A1	12-10-1994
		EP 0902034 A2	17-03-1999
		EP 0953835 A1	03-11-1999
		ES 2058921 T3	01-11-1994
		ES 2129101 T3	01-06-1999
